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ORIGINAL SUBMISSION

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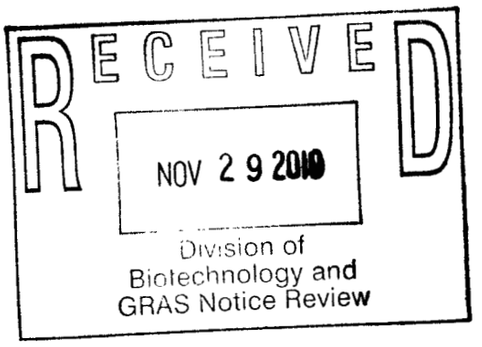
东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.



Office of Food Additive Safety (HFS-255),
Center for Food Safety and Applied Nutrition,
Food and Drug Administration,
5100 Paint Branch Parkway,
College Park, MD 20740

Date: November 15, 2010

GRAS Notice for Levocarnitine

Dear Sir,
In accordance with proposed 21 CFR s170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the ***Federal Register*** [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Northeast Pharmaceutical Group Co., Ltd, No. 37, Zhonggong Bei Street, Tiexi District, Shenyang, Liaoning Province, P.R.China Postcode: 110026], a Notice of the determination, on the basis of scientific procedures, that levocarnitine, produced by Northeast Pharmaceutical Group Co., Ltd (NEPG), as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the ***Federal, Food, Drug and Cosmetic Act.*** Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of the levocarnitine of NEPG under the intended conditions of use, also are enclosed for review by the agency.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,
(b) (6)

Xie Dan

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No.37 Zhonggong Bei Street, Tiexi District, Shenyang City, Liaoning Province, P.R.China 110026

NEPG

GRAS Notice for Levocarnitine

November 2010

Prepared for: Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Prepared by: Northeast Pharmaceutical Group Co., Ltd
No. 37, Zhonggong Bei Street,
Tiexi District, Shenyang, Liaoning Province,
P.R.China Postcode: 110026

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东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

I. GRAS EXEMPTION CLAIM

Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §I 70.36(c)(1) [62 FR 18938 (1 7 April 1997)]

As defined herein, levocarnitine has been determined by Northeast Pharmaceutical Group Co., Ltd (NEPG) to be Generally Recognized as Safe (GRAS) consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act.* This determination is based on scientific procedures, as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of levocarnitine under the conditions of its intended use in food. Therefore, the use of levocarnitine in food as described below is exempt from the requirement of premarket approval.

Signed by

(b) (6)

Date: 2010. 11. 15

Quality department,
Northeast Pharmaceutical Group Co., Ltd
qa@negpf.com.cn

I. A Name and Address of Notifier

Northeast Pharmaceutical Group Co., Ltd (NEPG)
No. 37, Zhonggong Bei Street,
Tiexi District, Shenyang, Liaoning Province,
P.R.China Postcode: 110026

I. B Common name of notified substance

Levocarnitine

I. C Conditions of Intended Use in Food

NEPG intends to market Levocarnitine as a food ingredient in a variety of food, beverage and milk powder. The level of use of Levocarnitine is presented as below:

Chewing tablets, oral liquid, capsule: 250～600mg/tablet, piece, pill;

Milk powder: 300～400g/kg;

Fruit juice or fruit flavor beverage, milk beverage: 600～3000mg/kg【1】.

Formula milk powder for children: 5~15 mg/100g【2】.

Sports nutrition food: 1－4g/day 【3】.

I. D Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Levocarnitine has been determined by NEPG to be GRAS on the basis of scientific procedures. This GRAS determination is based on scientific data generally available in the public domain pertaining to the safety of Levocarnitine, as discussed herein, and on a consensus of opinion among a panel of experts qualified by scientific training and experience to evaluate the safety of Levocarnitine as a component of food [**Appendix A** Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Levocarnitine as a food Ingredient].

I. E Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Northeast Pharmaceutical Group Co., Ltd (NEPG)
No. 37, Zhonggong Bei Street,
Tiexi District, Shenyang, Liaoning Province,
P.R.China Postcode: 110026

Should the FDA have any questions or additional information requests regarding this notification, NEPG will supply these data and information in time.

II. L-CARNITINE CHEMISTRY AND COMPOSTION

II.A Identity

Levocarnitine is a white crystals or crystalline powder, hygroscopic; with slightly specific odour. Freely soluble in ethanol, water, alkali solution and dilute inorganic acid solution, practically insoluble in acetone and ethyl acetate.

Common or Usual Name: Levocarnitine

Formal Names (IUPAC or CAS Names): LEVOCARNITINE

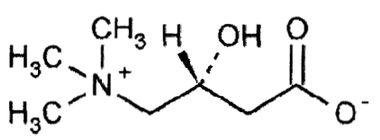
CAS Registry Number: 541-15-1

Chemical Formula: $C_7H_{15}NO_3$

Molecular weight: 161.20

Chemical name: (R)-3-Carboxy-2-hydroxy-N,N,N-trimethyl-1-propanaminium Hydroxide, inner salt

Structural Formula



II.B Method of Manufacture

Chemical synthesis method

3-chloro-2-hydroxypropyltrimethylammonium chloride (hereinafter referred to as L-quaternary ammonium salt) reacts with sodium cyanide to produce 3-cyano-2-hydroxy- propyltrimethylammonium chloride (hereinafter referred to as *intermediate*) via cyanidation. Subsequently, Levocarnitine is synthesized through hydrolyzation and addition reaction, and then ion-exchange and purification. The purified Levocarnitine is sieved, blended and sampled for analyses. Finally, the qualified product is packaged, and the final product of Levocarnitine is obtained for sale.

Step 1. Preparation of intermediate

1.1 Cyanidation

Dissolve sodium cyanide with drinking water. Transfer the obtained solution into the

storage tank. Introduce methanol and L-quaternary ammonium salt into the cyanidation reactor, and start to agitate, raise the temperature. Then add the sodium cyanide solution to the reactor.

After the reaction, add hydrochloric acid, adjust the pH value of the content and maintain the temperature for reaction, filter the reaction solution. Transfer the separated liquid to the concentration tank. Concentrate the liquid in the tank under reduced pressure until the liquid is dry.

1.2 Decolorization and purification

Add activated carbon and methanol into the concentration tank, Reflux and decolor. Filter and transfer the filtrate into the crystallization tank. Cool and crystallize. Centrifuge and dry the cake, then the intermediate is obtained.

Step 2. Preparation of Levocarnitine

2.1 Hydrolysis

Transfer the intermediate into the hydrolysis reactor, and add refined hydrochloric acid to the reactor. Start the agitator to dissolve. Raise the temperature and maintain the temperature for reaction. After that, concentrate the content under reduced pressure. Reduce the temperature and adjust the pH by adding concentrated ammonia to the reactor. Decrease temperature and filter. Transfer the filtrate and washings (hydrolysate) to the addition tank.

2.2 Addition reaction

Suck the prepared sodium metabisulfite solution to the addition tank containing hydrolysate. Add purified water to the tank, raise the temperature for reaction. Then transfer the content of the tank into the dilution tank, and add purified water.

2.3 Ion exchange

Load the diluted liquid to the cation exchanging column to conduct ion-exchange.

2.4 Concentration

After ion-exchange, concentrate the eluent under reduced pressure.

2.5 Decolorization and adsorption

Add activated charcoal to the tank. Remain the temperature and agitate for reaction. Filter the decolored liquid to the adsorbing tank. Then add the regenerated anion exchange resin to the tank. Allow to adsorb with agitating until the sample is qualified.

Filter and transfer the filtrate into the 2nd concentration tank.

2.6 Crystallization

Concentrate the above-mentioned liquor till dried.

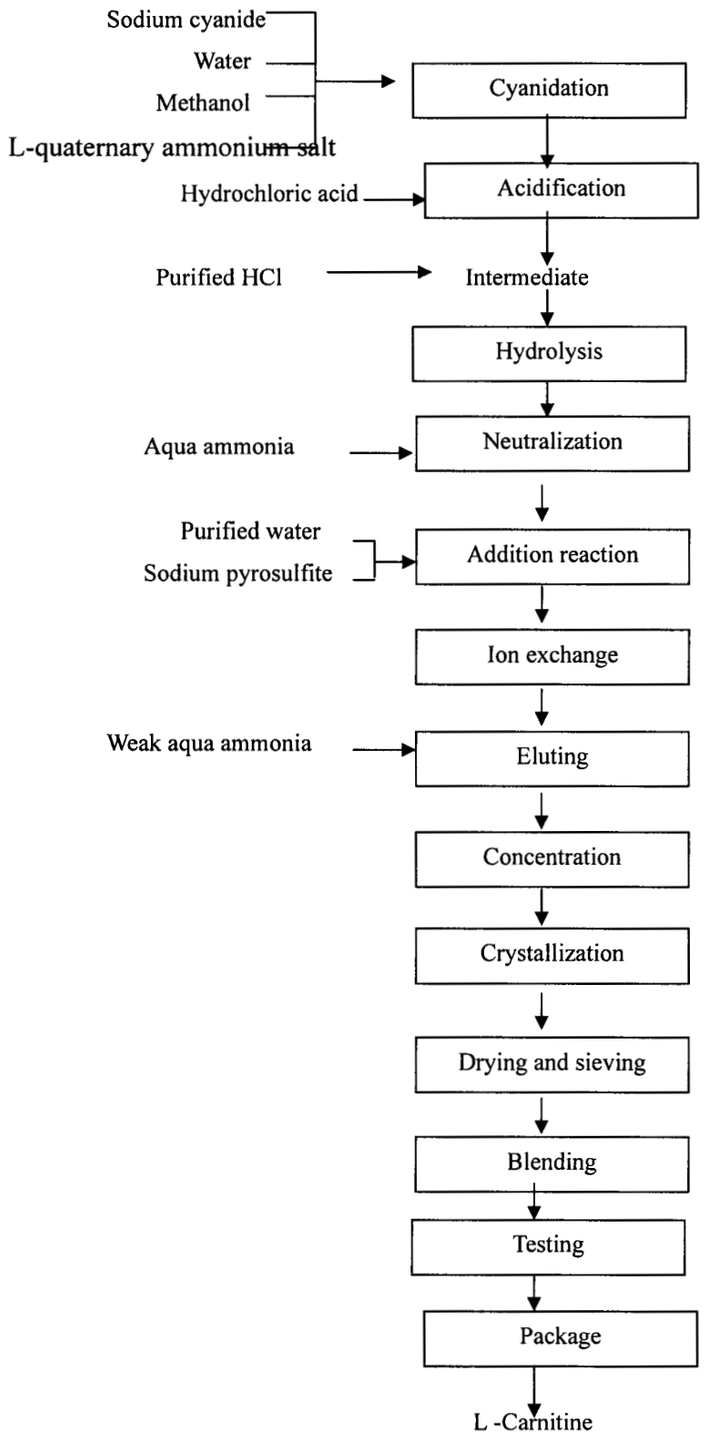
Add anhydrous ethanol and diatomite to the tank. Raise the temperature to reflux. After that, filter the content of the tank through a precision filter. Transfer the filtrate into the crystallizing tank, concentrate it until the crystals separate out, add acetone, stirring, and decrease temperature and crystallization. Then centrifuge to separate the materials, obtain wet levocarnitine.

2.7 Drying and blending

Transfer the wet levocarnitine into double cone dryer for drying, sieving. Then blend it, the final dried levocarnitine is obtained.

The schematic overview of manufacturing process of levocarnitine is presented in figure II. B-1

Figure II. B-1 Schematic Overview of the Manufacturing of Levocarnitine



II.C Specification of Food-grade material

II.C.1 Product Specifications

Levocarnitine is produced in accordance with current Good Manufacturing Practices (cGMP) and in order to ensure a consistent, safe product, NEPG has established specification parameters for the final product according to FCC7. These parameters are presented in Table II.C.l-1. Analysis of 5 consecutive, representative lots of levocarnitine was performed. These data are presented in the **Appendix B**.

Table II.C.l-1

No.	Test items	unit	FCC7
1	Appearance		White crystal or white crystalline hygroscopic powder
2	Identification		Positive
3	Assay	%	97.0 ~ 103.0
4	Optical rotation	°	-29.0 ~ -32.0
5	Acidity (pH)		5.5 ~ 9.5
6	Water content	%	≤4.0
7	Sodium	%	≤0.1
8	Chloride	%	≤0.4
9	Residue on ignition	%	≤0.5
10	Potassium	%	≤0.2
11	Lead	%	≤0.0001

II.C.2 Method of analyses

Monograph of l-carnitine in FCC7

DESCRIPTION

L-carnitine occurs as white crystals or as a white, crystalline, hygroscopic powder. It is freely soluble in water, in alcohol, in alkaline solutions, and in dilute mineral acids. It is practically insoluble in acetone and in ethyl acetate. It decomposes without melting at about 185° to 195°.

IDENTIFICATION

- A. PROCEDURE

Analysis: Dissolve 1 g of sample in 10mL of water and 10mL of 1 N hydrochloric acid, and add 5 Ml of sodium tetraphenylborate TS.

Acceptance criterion: A white precipitate forms.

- B. INFRARED ABSORPTION, Spectrophotometric identification tests, appendix IIIC

Reference standard: USP Levocarnitine RS

Sample and standard preparation: K(previously dried in vacuum at 60° for 5 h)

Acceptance criterion: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *reference standard.*

ASSAY

- PROCEDURE

Sample: 1.0 g [avoid atmospheric moisture uptake during weighing.]

Analysis: Dissolve the sample in water contained in a 250-mL flask. Titrate with 1.0 N hydrochloric acid to a potentiometric endpoint. Perform a blank determination (see general provisions), and make any necessary correction. Each mL of 1.0 N hydrochloric acid is equivalent to 161.2 mg of $C_7H_{15}NO_3$.

Acceptance criteria: NLT 97.0% and NMT 103.0% of $C_7H_{15}NO_3$, calculated on the anhydrous basis.

IMPURITIES

Inorganic impurities

- CHLORIDE

Sample solution: Add 100mg of sample in 30 to 40 mL of water in a 50-mL flask, and mix. Add 10% nitric acid dropwise until the solution is neutral to litmus. Add an additional 1 mL of 10% nitric acid and dilute with water to a total volume of 50 mL.

Control: Add 0.56mL of 0.02 N hydrochloric acid solution to 30 to 40 mL of water in a 50-mL flask. Add 1mL of 10% nitric acid and dilute to volume with water.

Analysis: Add 1 mL of 0.1 N silver nitrate to both the sample solution and the control. Mix, allow to stand for 5 min protected from direct sunlight, and visually compare the two solutions.

Acceptance criterion: Any turbidity produced by the sample solution does not exceed that shown in the control (NMT 0.4%).

- LEAD, lead limit test, flame atomic absorption Spectrophotometric method, appendix IIIB

  Sample: 10g

  Acceptance criterion: NMT 1mg/kg

- POTASSIUM

[Note: The standard solution and the sample solutions may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the spectrophotometer.]

Standard stock solution: 12.5mg/mL potassium, made by transferring 5.959 mg of potassium chloride, previously dried at 105° for 2h, into a 250-mL volumetric flask, dilute to volume with water, and mix.

Standard solution: 31.25 μg/mL potassium: from standard stock solution.

Sample: 62.5mg

Sample stock solution: Transfer the sample into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Sample solution: Add 0, 2.0, and 4.0mL of the standard solution to three separate 25-mL volumetric flasks. Add 20.0mL of the sample stock solution to each flask, dilute to volume with water, and mix. These solutions contain 0 (sample solution A), 2.5 (sample solution B), and 5.0 (sample solution C) μg/mL of potassium.

Analysis: Using a suitable atomic absorption spectrophotometer equipped with an air-acetylene flame and using water as the blank, concomitantly determine the absorbance values of the sample solutions at the potassium emission line at 766.7 nm. Plot the absorbance values of the sample solutions versus their contents of potassium, in μg/mL; draw the straight line best fitting the three points and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in μg, of potassium in each mL of sample

solution A. Calculate the percent potassium in the portion of sample taken by multiplying the concentration, in µg/mL, of potassium found in sample solution A BY 0.2.

Acceptance criterion: NMT 0.2%.

- SODIUM

[NOTE: The standard solution and the sample solutions may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the spectrophotometer.]

Standard stock solution: 10.0mg/mL sodium, made by transferring 6.355 g of sodium chloride, previously dried at 105° for 2h, into a 250-mL volumetric flask, dilute to volume with water, and mix.

Standard solution: 250 µg/mL sodium: from standard stock solution.

Sample: 4g

Sample stock solution: Transfer the sample into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Sample solution: Add 0, 2.0, and 4.0mL of the standard solution to three separate 25-mL volumetric flasks. Add 20.0mL of the sample stock solution to each flask, dilute to volume with water, and mix. These solutions contain 0 (sample solution A), 20.0 (sample solution B), and 40.0 (sample solution C) µg/mL of sodium.

Analysis: Using a suitable atomic absorption spectrophotometer equipped with an air-acetylene flame and using water as the blank, concomitantly determine the absorbance values of the sample solutions at the sodium emission line at 589.0 nm. Plot the absorbance values of the sample solutions versus their contents of sodium, in µg/mL; draw the straight line best fitting the three points and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in µg, of sodium in each mL of sample solution A. Calculate the percent sodium in the portion of sample taken by multiplying the concentration, in µg/mL, of sodium found in sample solution A by 0.003125.

Acceptance criterion: NMT 0.1%.

SPECIFIC TESTS

- OPTICAL (SPECIFIC) ROTATION, appendix IIIB

Sample solution: 100mg/mL (using a previously dried sample)

Acceptance criterion: $[\alpha]_D^{20}$ Between -29.0° and -32.0°, calculated on the anhydrous basis

- pH, Ph determination, appendix IIIB

Sample solution: 50 mg/mL

Acceptance criterion: Between 5.5 and 9.5

- RESIDUE ON IGNITION (sulfated ash), appendix IIC

Sample: 2g

Acceptance criterion: NMT 0.5%

- WATER, water determination, appendix IIB

Acceptance criterion: NMT 4.0%

II.C.3 Stability of levocarnitine

The stability of Levocarnitine has been evaluated under numerous conditions.

-Long-term testing: Under the condition of temperature at 25±2°C and a relative humidity (RH) at 60±5%, the samples were tested at the beginning of the test and at the end of the 3rd, 6th, 9th, 12th, 18th and 24th month on the items of characters, identification, specific rotation, pH, water content, assay and related substances.

-Accelerated testing: Under the condition of a temperature at 40±2°C and a relative humidity (RH) at 75±5%, the samples were tested at the beginning of the test and at the end of the 1st, 2nd, 3rd, 4th, 5th and 6th month on the items of characters, identification, specific rotation, pH, water content, assay.

The above two testing was performed since April 2008. See **appendix C** for the testing data. The results show that all the examined items meet the requirement of specifications for Levocarnitine established by the company when stored in the same package as that of commercial batches over the whole period of stability testing.

-Stress testing

This testing was performed in 2004. So the batches are different from the above two testing. See table II.C.3-1 for the testing data.

Normal temperature (NT): Under the condition of a temperature at 25±2°C and a relative humidity (RH) at 60±5%; prepare the test solution before use.

Photolysis (P): The sample is exposed to light providing an overall illumination of 4500 lux, and tested at the beginning of the test and at the end of the 3rd, 5th and 10th day; prepare the test solutions before use.

Treated with water (WT): Weigh 1.25 g of the substance to be examined to a 50 ml volumetric flask, dissolve with purified water and dilute to volume. Mix well and allow to stand for 24 hours. After that, transfer 5.0 ml of the solution to a 25 ml volumetric flask, dilute to volume with mobile phase(a mixture of 35 volumes of 6.81 g/L solution of potassium dihydrogen phosphate adjusted to pH 4.7 with dilute sodium hydroxide solution, and 65 volumes of acetonitrile) and mix well.

Treated with acid (AcT): Weigh 1.25 g of the substance to be examined to a 50 ml

volumetric flask, dissolve with 20 ml of 10% hydrochloric acid solution and allow to stand for 24 hours. After that, neutralize with 10% sodium hydroxide solution and dilute to volume with purified water. Mix well. Transfer 5.0 ml of the solution to a 25 ml volumetric flask, dilute to volume with mobile phase and mix well.

Treated with alkali (AlT): Weigh 1.25 g of the substance to be examined to a 50 ml volumetric flask, dissolve with 20 ml of 10% sodium hydroxide solution and allow to stand for 24 hours. After that, neutralize with 10% hydrochloric acid solution and dilute to volume with purified water. Mix well. Transfer 5.0 ml of the solution to a 25 ml volumetric flask, dilute to volume with mobile phase and mix well.

High temperature (HT): Under the condition of a temperature at 60°C, and tested at the beginning of the test and at the end of the 5^{th} and 10^{th} day; prepare the test solutions before use.

- Conclusion:

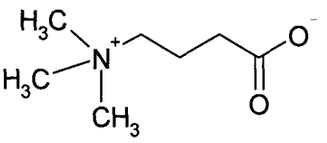
From the results of stress testing, it is clear that any new impurity is not detected during the whole period of all the tests. So it can be concluded that Levocarnitine is stable to light, water, acid and heat. However, under the existence of alkali, Levocarnitine degrades a little, and the content of (E)- or (Z)-4-(trimethyl-ammonio)but-2-enoate increased.

Table II.C.3-1

Retention time / Batch No.	Peak areas, %	
	10.7 min (Levocarnitine)	12.1 min ((E)- or (Z)-4-(trimethyl-ammonio)but-2-enoate)
200408201 (NT)	99.64	0.31
200408202 (NT)	99.44	0.48
200408203 (NT)	99.56	0.37
200408201 (P, 1st day)	99.78	0.16
200408202 (P, 1st day)	99.70	0.26
200408203 (P, 1st day)	99.73	0.25
200408201 (P, 3rd day)	99.66	0.28
200408202 (P, 3rd day)	99.63	0.27
200408203 (P, 3rd day)	99.69	0.30
200408201 (P, 5th day)	99.69	0.28
200408202 (P, 5th day)	99.60	0.33
200408203 (P, 5th day)	99.94	0.05
200408201 (P, 10th day)	99.53	0.42
200408202 (P, 10th day)	99.69	0.26
200408203 (P, 10th day)	99.65	0.32
200408201 (WT)	99.53	0.42
200408202 (WT)	99.57	0.43
200408203 (WT)	99.71	0.28
200408201 (AcT)	99.51	0.41
200408202 (AcT)	99.50	0.40
200408203 (AcT)	99.61	0.29
200408201 (AlT)	98.30	1.63
200408202 (AlT)	98.06	1.78
200408203 (AlT)	98.50	1.50
200408201 (HT 5th day)	99.61	0.38
200408202 (HT 5th day)	99.60	0.37
200408203 (HT 5th day)	99.69	0.28
200408201 (HT 10th day)	99.60	0.39
200408202 (HT 10th day)	99.58	0.41
200408203 (HT 10th day)	99.68	0.31

Possible organic impurities

- (E)- or (Z)-4-(trimethylammonio)but-2-enoate

 and (Z)-isomer

(E)- or (Z)-4-(trimethylammonio)but-2-enoate is a by-product, mainly obtained from the hydrolysis reaction of the second intermediate in hydrochloric acid. The following addition reaction and ion exchange can remove most of (E)- or (Z)-4-(trimethylammonio)but-2-enoate. Another possible way of producing this impurity is the Step 3.4 Concentration. The ammonia makes the solution alkali at the beginning, which would result in a trace quantity of (E)- or (Z)-4-(trimethylammonio)but-2-enoate. While the following purification step may reduce the residual quantity. (E)- or (Z)-4-(trimethylammonio)but- 2-enoate is controlled to each batch of Levocarnitine. Considering the results of the former batch release tests, content of residual (E)- or (Z)-4-(trimethylammonio)but-2-enoate is always within the prescribed limit (not more than 0.50%, the limit in the monograph for Levocarnitine in Ph. Eur.6.0 is referenced).

III. SELF-LIMITING LEVELS OF USE

Levocarnitine is a naturally attacking substance required in mammalian energy metabolism. There have been no reports of toxicity from levocarnitine overdosage. Levocarnitine is easily removed from plasma by dialysis. When using the levocarnitine in food and beverage, please refer to *I. C Conditions of Intended Use in Food* for limiting levels.

IV. BASIS FOR GRAS DETERMINATION

IV.A Documentation to Support the Safety of Levocarnitine

The determination that Levocarnitine is GRAS is on the basis of scientific procedures. The safety of Levocarnitine under the intended conditions is based on the estimate of the probable consumption of the ingredient, an extensive amount of published scientific data demonstrating the endogenous presence of Levocarnitine in humans and publicly available pharmacokinetic studies, toxicity studies, and studies in humans of Levocarnitine.

These data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of Levocarnitine as a component of food, who concluded that the proposed uses of Levocarnitine are safe and suitable and would be GRAS based on scientific procedures [see Appendix A, entitled, "Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Levocarnitine as a food Ingredient"] and that other qualified experts would concur with these conclusion. It also is NEPG's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. A summary of these data is presented herein.

IV.B Probable Consumption of Levocarnitine

Animal products like meat, fish, poultry, and milk are the best sources. Dairy products contain carnitine primarily in the whey fraction 【4,5,6】. The carnitine content of several foods is listed in Table IV. B-1.

Table IV. B-1: **Selected food sources of carnitine** 【4】

Food	Milligrams (mg)
Beef steak, cooked, 4 ounces	56-162
Ground beef, cooked, 4 ounces	87-99
Milk, whole, 1 cup	8
Codfish, cooked, 4 ounces	4-7
Chicken breast, cooked, 4 ounces	3-5
Ice cream, ½ cup	3
Cheese, cheddar, 2 ounces	2
Whole-wheat bread, 2 slices	0.2
Asparagus, cooked, ½ cup	0.1

A well-balanced, non-vegetarian Western diet is estimated to provide 100-300 mg of L-Carnitine each day【7】. In Europe, however, dietary L-Carnitine intake has decreased by about 20% over the last decade, mainly as a result of a decrease in beef consumption.

Table 2: Average dietary L-Carnitine intake in various countries [mg/day]

Country	L-Carnitine mg /day
Mongolia	425
Australia	301
US	236
Europe	129
Japan	75
India	24

The average non-vegetarian diet provides up to 100 mg L-carnitine daily, or up to 300 mg in high meat eaters【8】(Borum PR. Carnitine. Ann Rev Nutr 1983, 3: 233-259; Lennon et al., 1986; Feller AG et al. Role of carnitine in human nutrition. J Nutr 1988, 118: 541-547). The richest sources are from meat, sheep muscle containing the most at around 207 mg/100 g, with milk, rice and bread being lesser sources【9】(Scholte HR et

al. Metabolism, Function and Transport of Carnitine in Health and Disease. In: Gitzelmann R et al (eds.) Carnitine in der Medizin. 1987, Schattauer Verlagsgesellschaft mbH Stuttgart, Germany). Carnitine homeostasis is maintained by absorption from dietary sources, a modest rate of biosynthesis and highly efficient reabsorption of carnitine in the kidney (Rebouche and Seim, 1998). Estimates of the amount of L-carnitine absorbed from the diet vary from about 30 - 40% (Harmeyer, 2000) to 54 - 87% (Rebouche and Seim, 1998).

No dietary reference intakes (DRIs) or recommended daily allowances (RDAs) have yet been set for L-Carnitine, because such values exist only for vitamins and minerals and other essential substances. L-Carnitine is regarded as a conditionally essential nutrient because in certain situations, the need may exceed the capacity for endogenous synthesis.

However, Hathcock【10】 recommends that intake up to 2000mg/day is safe evidenced with the observed safe level (OSL) risk assessment method.

Cruciani and Dvorkin, etc【11】think that l-carnitine may be safely administered at doses up to 3000 mg/day.

IV.C Endogenous Presence of Levocarnitine

L-Carnitine is supplied to the body through both endogenous synthesis and food intake. The human body synthesizes about 20 mg of L-Carnitine every day. The major sites of L-Carnitine biosynthesis are the liver and kidneys. Synthesis【12】 of carnitine begins with methylation of the amino acid L-lysine by S-adenosylmethionine (SAMe). Magnesium, vitamin C, iron, vitamins B3 and B6, and alpha-ketoglutarate – along with the cofactors responsible for creating SAMe (methionine, folic acid, vitamin B12, and betaine) – are all required for endogenous carnitine synthesis.

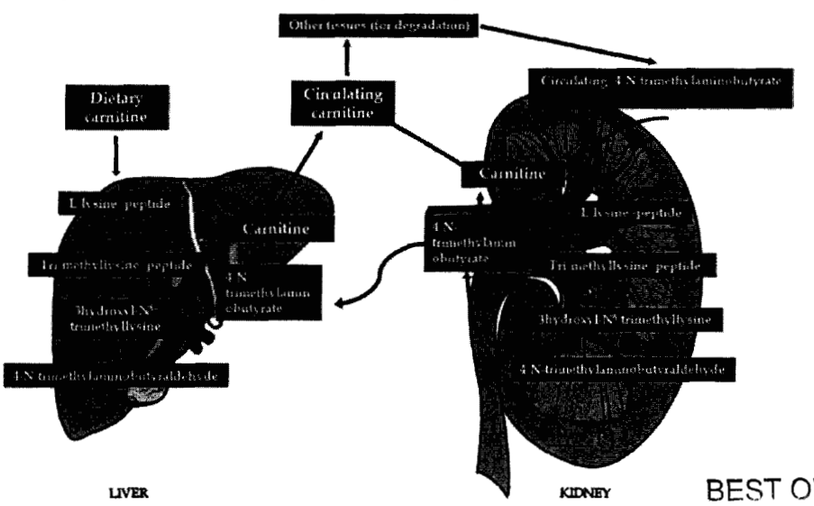
Evidence indicates L-carnitine is absorbed in the intestine by a combination of active transport and passive diffusion【13】. Reports of bioavailability following an oral dose

have varied substantially, with estimates as low as 16-18 percent【14,15】 and as high as 54-87 percent【16,17】.

IV.D Metabolism

IV.D.1 Carnitine biosynthesis and metabolism【18】

Carnitine, a branched non-essential amino acid, is synthesized from the essential amino acids lysine and methionine. Ascorbic acid, ferrous iron, pyroxidine and niacin are also necessary cofactors and deficiencies of any of these can lead to carnitine deficiency. The pathway in mammals is unique using protein-bound lysine that is enzymatically methylated to form trimethyllysine as a post-translational modification of protein synthesis. Trimethyllysine undergoes four enzymatic reactions in the course of endogenous L-carnitine biosynthesis (Figure **IV.D.1**-1).



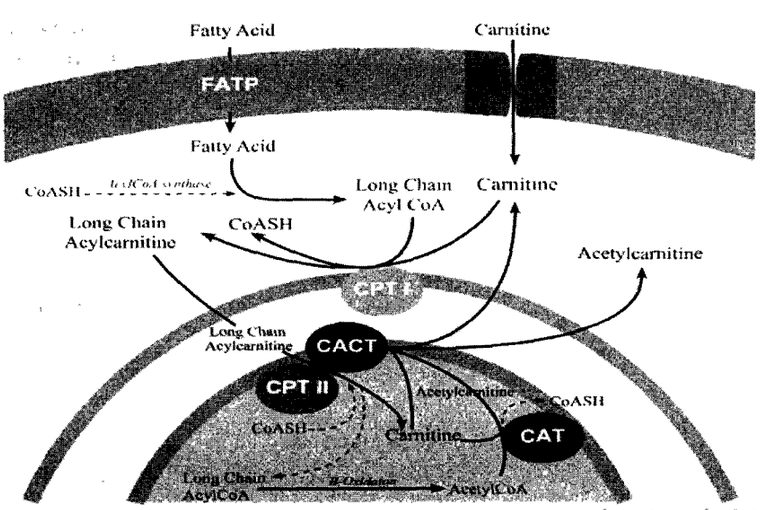
One of the enzymes in this pathway, γ-butyrobetaine hydroxylase, is absent from cardiac and skeletal muscle but highly expressed in human liver, testes, and kidney. The rate of L-carnitine biosynthesis in vegetarians is estimated to be around 1.2 μmol/kg of body weight/day. Omnivorous humans ingest 2-12 μmol/kg of body weight/day which represents 75% of body carnitine sources. Neither renal reabsorption nor changes in dietary carnitine intake appear to affect the rate of endogenous carnitine synthesis.

Bioavailability of oral carnitine dietary supplements is only in the order of 14 to 18% of dose and unabsorbed L-carnitine is mostly degraded by microorganisms in the large intestine.

Free L-carnitine, absorbed from dietary intake or synthesized in liver and kidney, reaches the blood stream and the extracellular fluid. Its transport within cells of various tissues is limited by their respective uptake capacities. Plasma concentration of free carnitine is in dynamic balance with acylcarnitines with the acyl to fee carnitine ration of ≤ 0.4 being considered normal. Acetylcarnitine esters are formed intracellularly during regular metabolic activity. Long chain acetylcarnitine esters transport fatty acyl moieties into the mitochondria (Figure **IV.D.1-2**). Short and medium-chain acetyl esters, formed in the mitochondria and peroxisomes, participate in the removal of organic acids. Acetyl-L-carnitine is the principal acylcarnitine ester. Acetyl-L-carnitine participates in both anabolic and catabolic pathways in cellular metabolism.

Figure IV.D.1-2

Carnitine is actively transported via OCTN2 into the cytosol to participate in the shuttling of activated long chain fatty acids into the mitochondria where β-oxidation takes place. Carnitine also regulates the Coenzyme A (CoA)/acylCoA ratio within mitochondria, modulation of which reduces accumulation of toxic acyl-CoA compounds and maintains energy production.



Carnitine plays a critical role in energy balance across cell membranes and in energy metabolism of tissues that derive much of their energy from fatty acid oxidation such as cardiac and skeletal muscles (Figure **IV.D.1-2**). Although carnitine plays its main role in carnitine free fatty acid metabolism, it also enhances carbohydrate utilization. Uptake in skeletal and cardiac muscle is a saturable active transport process against a concentration gradient.

Experimental evidence suggests that the transport of long chain fatty acids into the mitochondria is a rate limiting step in fatty acid oxidation. During sustained low to moderate exercise, fatty acid oxidation increases to become the predominant energy source to muscles. CPTI (Figure **IV.D.1**-2) is a control point of FA oxidation and decreased carnitine levels and acidosis of CPT1 have been implicated in decreased fatty acid oxidation during heavy exercise. Deficiencies in CPTII may result in exercise induced muscle injury due to inability to increase FA oxidation with increased exertion. Carnitine participates in cell volume and fluid balancing in all tissues that are affected by the tonicity (iso-, hyper- hypo- tonicity) of the extracellular environment. Data suggest that despite fluctuations in carnitine concentration due to its osmolytic pressure changes, carnitine maintains its energy production capacities and often osmolytic gradients can be harnessed for energy. Carnitine fluctuates with both physiological and pathological changes in osmotic pressure. In one example of a physiological response to osmotic pressure, in early mammary gland milk production osmoregulatory pathways are exploited using asymmetric kinetics to increase the carnitine concentration in milk for suckling neonates who have reduced carnitine stores, even though this results in decreased maternal liver stores.

IV.D.2 Absorption, Distribution, Metabolism and Excretion (ADME) Date【19】

IV.D.2.1 Absorption and Plasma Concentration

The extent and rate of absorption of oral or intramuscular l-carnitine has not been adequately studied. After a 500mg oral dose, the mean maximal concentration in the plasma of healthy volunteers was 48.5μmol/L attained at an average of 5 hours (Kudoh et al. 1984).

Similarly, in 12 volunteers given a single 2g dose of oral l-carnitine, the maximal

concentrations of free and total l-carnitine were 58μmol/L and 69μmol/L, respectively, at 3.5 hours after the dose (Bach et al. 1983). In comparison, normal values for the total endogenous compound have been found to be 57μmol/L for men and 46μmol/L for women who were healthy but obese (Cederblad 1976), and 50μmol/L and 39μmol/L for healthy men and women, respectively, who were not obese (Fagher et al. 1985b).

Hamilton et al.(1986) demonstrated in human intestinal mucosal specimens obtained by biopsy, that l-carnitine is actively transported via a saturable system against a concentration gradient in the duodenum and ileum, but not the colon, and

That passive diffusion also occurs. The rate of transport of l-carnitine into muscle is decrease in patients with primary carnitine deficiencies (Rebouche & Engel 1984).

IV.D.2.2 Distribution

Investigations with intravenously administered dl-carnitine or radiolabelled 1-methyl 3H-carnitine have shown that the apparent volume of distribution of l-carnitine using a 2-compartment model is about 26% expressed as percent of bodyweight (Cederblad 1984; Welling et al. 1979). Welling et al. (1979) demonstrated that in cardiac patients given dl-carnitine 40 to 60 mg/kg intravenously, the compound was rapidly distributed initially into about 20% of bodyweight, with later redistribution to a volume of 30%.

With the exception of an in vitro study by Schmidt-Sommerfeld et al. (1985), which showed that l-carnitine diffuses passively from the maternal to the fetal circulation but is actively transported into the placenta. No studies have specifically investigated the distribution of exogenous l-carnitine into various human tissues.

IV.D.2.3 Elimination

IV.D.2.3.1 Metabolism and Excretion

In studies in rats in vivo and in vitro, greater than 95% of a radiolabelled dose of l-carnitine given intravenously or intraperitoneally is retrievable in the urine and faeces as unaltered drug (Gross & Henderson 1984; Mehlman et al. 1969; Rebouche et al. 1984). In contrast, Rebouche et al. (1984) found that after an oral dose, only 34% appeared as unchanged l-carnitine with the remainder recovered in the urine as trimethylamine N-oxide. These investigators concluded that the extent of metabolism

was related to the size of the dose, and that endogenous intestinal flora was responsible for the metabolism of l-carnitine taken orally.

Metabolic studies in man are lacking. Rebouche and Engel (1984) found no significant conversion of l-carnitine to metabolites in healthy subjects or patients with systemic carnitine deficiencies; acylcarnitine esters comprised 20% of the recovered dose in plasma and 43% in urine. Moreover, faecal excretion accounts for less than 2% of the elimination of l-carnitine (Rebouche & Engel 1984). Thus, the compound is likely eliminated virtually unchanged via the kidneys.

In studies of excretion, the portion of l-carnitine administered as dl-carnitine or l-carnitine which is recoverable in the urine is dependent upon the route of administration. In a study of 15 middle-aged cardiac patients given t40 or 60 mg/kg of dl-carnitine by the intravenous infusion about 70% (range 11.6 to 127%) was retrieved in the urine within 12 hours and 80% in 24 hours (Welling et al. 1979).

In contrast, Bach et al. (1983) recovered only 7% of an oral 2g dose in the urine of 12 volunteers within 24 hours, which is similar to the 10% reported by Suzuki et al. (1976) in students following an oral 500mg dose of dl-carnitine. Moreover, Frohlich et al. (1978) reported a 10% increase in the excretion of l-carnitine during the first 6 hours after administration of a 1g oral dose in volunteers, compared with excretion in untreated control subjects.

IV.D.2.3.2 Half-life

In healthy volunteers and in patients with coronary artery disease, the half-life of l-carnitine has varied from about 2 to 15 hours, following single oral or intravenous doses of dl or l-carnitine 500mg to 2g (Bach et al. 1983; Cederblad 1984; Kudoh et al. 1984; Welling et al. 1979).

IV.E Preclinical Studies Pertaining to the Safe Consumption of Levocarnitine

NEPG commissioned laboratory belongs to Liaoning Province Center for disease control and prevention to perform animal tests according to *food safety toxicity evaluation procedure and method*(*GB15193-2003*) in July 2010, with levocarnitine batch number of DY017100142, including the acute oral toxicity test in mice,

Micronucleus Test of Mice Bone Marrow Polychromatic Erythrocytes and AMES test. Being through two months tests, obtain the following results which demonstrate that:

1. An acute oral toxicity test in mice: the female and male mice LD_{50}>15000mg/kg BW, which shows no toxicity of levocarnitine.
2. Micronucleus Test of Mice Bone Marrow Polychromatic Erythrocytes: the various dose groups are compared with control groups, there is no obvious difference on the micronucleus cell rate, and the test result is negative.
3. Salmonella typhimurium/mammal microsomal enzyme test (AMES test): the test result is negative.

See below for details.

IV.E.1 Acute Toxicity Study

Method:

20 healthy white mice with 18-22g(female and male occupies 50% respectively). Fill the mice by gastric gavage with the maximum tolerated dose. Starving the mice for 16hours before test (overnight), water is permitted to feed. Prepare the levocarnitine solution as 0.25g/mL, perfuse three times every 8 hours, volume at 20ml/kg BW, dose at 15000 mg/kg BW. After gastric perfusion, observe for 14 days, record the poisonous syndrome and death toll.

Result:

When fill the mice levocarnitine 15000mg/kg BW, no abnormal reaction during the observation duration, normal appetite, no death (see below table IV.E.1-1). When test the body of death mice, no abnormal effect was found. Therefore, LD_{50} is more than 15000mg/kg BW. The acute oral toxicity test shows that levocarnitine is nonpoisonous to mice.

Table IV.E.1-1 test results of acute oral toxicity of levocarnitine to mice

Gender	Dose(mg/kg)	quantity	Body weight, g			Death toll	Death rate (%)
			0d	7d	14d		
♂	15000	10	21.2±0.70	32.7±0.74	39.2±5.66	0	0
♀	15000	10	20.7±1.06	28.0±2.67	32.4±2.36	0	0

IV.E.2 Mutagenicity Data

IV.E.2.1 Information from literature

Mutagenicity data indicate no mutagenicity【12】; however, experiments to determine long-term carcinogenicity have not been conducted ...

/ALTERNATIVE and IN VITRO TESTS/【20】 In Hela cells, L-carnitine reduced glucocorticoid receptor-alpha affinity for its steroid ligand, and triggered nuclear translocation of the receptor. It suppressed glucocorticoid receptor-mediated tumor necrosis factor-alpha and interleukin-12 release by human primary monocytes stimulated with lipopolysaccharide ex vivo. All the these effects of L-carnitine were concentration dependent.

/ALTERNATIVE and IN VITRO TESTS/【21】 ... Whether addition of L-carnitine altered the tumor cytotoxic effects of epirubicin /was determined/ using a number of in vitro cell viability assays in different breast cancer cell lines including BT549, MDA-MB-435, NCI-ADR-RES, MCF7 and T47D. Additionally ... the ability of cells to respond to L-carnitine following analysis of the expression of carnitine metabolic enzymes by RT-PCR /was investigated/. ... Supplementation with L-carnitine had no effect on the ability of epirubicin to kill a variety of breast cancer cell lines. Additionally, no differences in the induction of apoptosis by epirubicin were observed. Furthermore, all cell lines examined expressed proteins required for carnitine uptake and use. ... These results suggest that supplementation with L-carnitine in patients undergoing epirubicin treatment could be safely used to reduce associated cardiotoxicities without fear that the efficacy of chemotherapy is jeopardized.

IV.E.2.2 Mutagenicity Data (in vitro)

Test bacteria: Salmonella typhimurium strains with histidine deficiency, TA97, TA98, TA100, TA102. The rats liver slurry (identified by biological activity) induced by phenobarbital sodium and β-napthtoflavone is as the vitro metabolic activation system (S_9).

Method: divide sample (levocarnitine) into five dose groups, 5.000mg/plate, 1.000 mg/ plate, 0.200 mg/ plate, 0.040 mg/ plate, 0.008 mg plate, the concentration of sample solutions are 50 mg/mL, 10 mg/mL, 2 mg/mL, 0.4 mg/mL, and 0.08 mg/mL. Set up the positive control group and solvent control group. Add 0.1ml of test bacteria increasing solution, 0.1ml of sample solution and 0.5ml of S_9 mix in the top layer culture medium. After mixing, pour it into the bottom plate, three plates for each dose. Culture it for 48hours at 37±1°C, record the reverse mutation bacterial colonies of each plate at various doses, and calculate the average value and standard deviation. The reverse mutation bacterial colonies obtained from the various dose groups is as twice or more than that from solvent control group, and if the dose-reaction relationship is formed, then determine that the sample can lead to mutagenicity.

Results: the reverse bacterial colonies of Salmonella typhimurium strains with histidine deficiency, TA97, TA98,TA100, TA102 are shown in the table IV.E.2.1-1 (first test) and table IV.E.2.1-2 (second test). The reverse bacterial colonies from positive control groups are obviously higher than that of solvent control groups (≥ two times); but the reverse bacterial colonies of various doses groups with or without liver microsomal enzyme activation system don't exceed one time that of solvent control groups, furthermore, no dose-reaction relationship is established. The test results indicate that this AMES test is negative.

Table IV.E.2.1-1 The first reverse mutation bacterial colonies results (average value ± standard deviation)

Groups	Dose	TA97		TA98		TA100		TA102	
	mg/plate	+ S_9	- S_9	+ S_9	- S_9	+ S_9	- S_9	+ S_9	- S_9
Levocarnitine sample	0.008	145±11	136±9	34±1	32±2	154±18	151±10	267±10	251±17
	0.040	160±17	148±12	41±4	34±3	152±16	148±18	247±18	241±14
	0.200	150±14	135±13	38±5	35±6	164±13	149±13	254±16	242±15
	1.000	148±12	143±14	35±3	29±4	157±14	139±10	251±21	244±16
	5.000	144±16	137±15	36±5	32±2	164±15	153±12	250±14	240±12
Solvents control group		148±14	128±12	36±3	33±4	163±16	141±11	251±19	237±17
Untreated control group		156±17	145±16	35±2	29±6	158±18	150±16	261±11	240±15
2-aminofluorene	0.010	1150±106		1387±93		546±71			
1, 8-dihydroxyanthraquinone	0.050							749±96	
dexon	0.050		2243±133		667±46				847±51
Sodium azide	0.0015						2097±144		

0000031

Table IV.E.2.1-2 The second reverse mutation bacterial colonies results (average value ± standard deviation)

Groups	Dose	TA97		TA98		TA100		TA102	
	mg/plate	+ S_9	- S_9	+ S_9	- S_9	+ S_9	- S_9	+ S_9	- S_9
Levocarnitine sample	0.008	145±12	140±13	34±5	27±4	153±14	147±15	253±15	247±13
	0.040	145±20	125±11	36±6	30±2	158±19	149±20	249±20	242±18
	0.200	147±17	144±17	39±3	35±4	155±12	148±14	261±10	237±13
	1.000	146±18	133±16	34±1	32±2	154±16	139±10	259±18	235±11
	5.000	161±11	145±14	35±5	31±3	157±13	151±13	254±17	247±14
Solvents control group		155±17	145±16	37±2	33±3	162±11	144±14	260±16	247±12
Untreated control group		152±14	125±10	36±5	31±2	156±18	151±16	253±19	242±16
2-aminofluorene	0.010	1093±112		1250±113		564±58			
1, 8-dihydroxyanthraquinone	0.050							747±67	
dexon	0.050		2220±123		696±75				726±50
Sodium azide	0.0015						2330±128		

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IV.E.2.3 Micronucleus Test of Mice Bone Marrow Polychromatic Erythrocytes

Method: Randomly divide 50 white mice with weight of 25-30g into five groups, 10 mice for each group, female and male possess a half. Set up three doses groups of 10000, 5000, 2500mg/kg BW, the distilled water is as the solvents control group, Cyclophosphamide is as the positive control (40mg/kg BW). The sample concentrations are 500mg/ml, 250 mg/ml, 125 mg/ml respectively, dilute with distilled water to the needed concentrations. Fill volume is 20mg/kg BW with the gastric perfusion method, perfuse once every 24 hours for two times. 6hours after the last perfusion, execute the mice to death, take out the breastbone marrow, and dilute it with calf serum, colorate it by Giemsa method, fixed by methanol in advance. Observe it with biological microscope, count each mouse 1000 polychromatic erythrocytes (PCE), the micronucleus rate is based on the PCE permillage of micronucleus. Count the Normochromatic Erythrocyte (NCE) observed from 200 PCE, calculates the PCE/NCE, and checks it with chi-square test.

Test results: see table IV.E.3-1. The PCE/NCE of each dose group is at 1.34-1.40, no obvious restrain effect on the mice bone marrow cells was observed. There is no obvious difference ($P>0.05$) of the micronucleus rate between various doses groups and solvent control group. However, here is obvious difference ($P<0.01$) of the micronucleus rate between Cyclophosphamide group and the solvent control group. No obvious damage side effect on the mice bone marrow cell chromosome was observed by levocarnitine.

Table IV.E.3-1 Results of micronucleus test of mice bone marrow polychromatic erythrocytes

gender	Dose mg/kg BW	Amount of animals	Observed PCE	Contained PCE	Micronucleus rate ‰	P value	Observed PCE	--PCE/NCE X ± SD	
male	10000	5	5000	7	1.40	1.00	1000	1.34	0.07
	5000	5	5000	7	1.40	1.00	1000	1.34	0.09
	2500	5	5000	7	1.40	1.00	1000	1.35	0.12
	Solvent control	5	5000	7	1.40		1000	1.33	0.09
	Positive control	5	5000	135	27.00	<0.01	1000	1.16	0.12
female	10000	5	5000	8	1.60	0.81	1000	1.40	0.09
	5000	5	5000	8	1.60	0.81	1000	1.38	0.05
	2500	5	5000	9	1.80	1.00	1000	1.40	0.09
	Solvent control	5	5000	9	1.80		1000	1.34	0.14
	Positive control	5	5000	138	27.60	<0.01	1000	1.17	0.10

Note: P is the testing probability value of X^2.

IV.F Studies in Humans

Müller DM and Seim H, etc. conducted the clinical study【22】, observe the effects of oral L-carnitine supplementation on in vivo long-chain fatty acid oxidation by measuring 1-[(13)C] palmitic acid oxidation in healthy subjects before and after L-carnitine supplementation (3 x 1 g/d for 10 days). They observed a significant increase in (13)CO(2) exhalation. That indicates that oral L-carnitine supplementation results in an increase in long-chain fatty acid oxidation in vivo in subjects without L-carnitine deficiency or without prolonged fatty acid metabolism.

Cruciani performed the clinical study to evaluate the safety and tolerability of l-carnitine【23】:

... a Phase I/II open-label trial to assess the safety and tolerability of exogenous L-carnitine and clarify the safe dose range associated with symptom effects /was conducted/ ... . Adult patients with advanced cancer, carnitine deficiency (free carnitine <35 for males or <25 uM/L for females, or acyl/free carnitine ratio >0.4), moderate to severe fatigue, and a Karnofsky Performance Status (KPS) score > or =50 were entered by groups of at least three into a standard maximum tolerated dose design. Each successive group received a higher dose of L-carnitine (250, 750, 1250, 1750, 2250, 2750, 3000 mg/day, respectively), administered in two daily doses for 7 days. To compare symptom outcomes before and after supplementation, patients completed validated measures of fatigue (Brief Fatigue Inventory [BFI]), depressed mood (Center for Epidemiologic Studies Depression Scale [CES-D]), quality of sleep (Epworth Sleeplessness Scale [ESS]), and KPS at baseline and 1 week later. Of the 38 patients screened for carnitine levels, 29 were deficient (76%). Twenty-seven patients participated ("intention to treat, ITT") (17 males, 10 females), and 21 completed the study ("completers"); 17 of these patients ("responders," mean+/-[SD] age=57.9+/-15) had increased carnitine levels at the end of the supplementation period. The highest dose achieved was 3000 mg/day. No patient experienced significant side effects and no toxicities were noted...

In role of carnitine in disease【18】, the authors cited a huge amount of clinical literatures to demonstrate the therapeutic effect of l-carnitine. L-carnitine can be use to cure primary and secondary levocarnitine deficiency, cardiovascular disease, type 2 diabetes, liver and kidney disease, Neuromuscular disease, Secondary genetic carnitine deficiency, Acquired carnitine deficiency, Obesity, endocrine disorders and diabetes, Dry eye and retinal disorders.

We did the clinical study on the levocarnitine produced by our company in June 1994-April 1995 to observe its therapeutic effects on angina pectoris and heart failure. Single blind, the placebo comparison method is used with169 patients. The research indicates that levocarnitine associating with other medicines of anti-angina, the clinical efficacy on ischemic heart disease and angina is 89.6%, has an effect on unstable and

fatigue angina, and could reduce attacking of angina. For 40 heart failure patients, 90% efficiency is reached to decrease heart rate, improve the syndrome and functions, increase exercise capacity. Refer to appendix D for the clinical study.

Side Effects

At doses of approximately 3 grams/day, carnitine supplements may cause nausea, vomiting, abdominal cramps, diarrhea, and a "fishy" body odor 【24，25】. More rare side effects include muscle weakness in uremic patients and seizures in those with seizure disorders.

IV.G Summary and Basis for GRAS Conclusion

The GRAS determination for Levocarnitine based on scientific procedures. Levocarnitine is manufactured in accordance with cGMP and meets appropriate food-grade specifications. The production process for Levocarnitine involves amination, cyanidation, hydrolyzation, addition reaction, and then ion-exchange and purification. The purified Levocarnitine is sieved, blended and sampled for analyses. Finally, the qualified product is packaged.

The solvents, such ethanol, methanol, acetone are used in the processing steps for Levocarnitine and/or are removed during the extensive purification processes, resulting in a final product of high purity (>97% levocarnitine).

Levocarnitine is present naturally in many foods, including fresh meat products and milk. The average non-vegetarian diet provides up to 100 mg L-carnitine daily, or up to 300 mg in high meat eaters. The richest sources are from meat, sheep muscle containing the most at around 207 mg/100 g, with milk, rice and bread being lesser sources. Carnitine homeostasis is maintained by absorption from dietary sources, a modest rate of biosynthesis and highly efficient reabsorption of carnitine in the kidney (Rebouche and Seim, 1998). Estimates of the amount of L-carnitine absorbed from the diet vary from about 30 - 40% (Harmeyer, 2000) to 54 - 87% (Rebouche and Seim, 1998).

Levocarnitine is an endogenous compound in humans and in animal species that serves an important carrier to transport of long chain fatty acids across the mitochondrial

membrane to the site of β-oxidation, resulting in the production of energy. Following oral administration, L-carnitine is absorbed in the intestine by a combination of active transport and passive diffusion【10】. Reports of bioavailability following an oral dose have varied substantially, with estimates as low as 16-18 percent【11,12】 and as high as 54-87 percent【13,14】.

The results of the animal and human studies of levocarnitine have been determined by NEPG to indicate that there is reasonable certainty that Levocarnitine is not harmful under the intended conditions of use. As levocarnitine is reported to be absorbed intestine, data from studies involving oral administration of levocarnitine were reviewed for the purpose of assessing the safety and GRAS status of Levocarnitine.

Randomly divide 50 white mice with weight of 25-30g into five groups, 10 mice for each group, female and male possess a half. Set up three doses groups of 10000, 5000, 2500mg/kg BW, Fill volume is 20mg/kg BW with the gastric perfusion method, perfuse once every 24 hours for two times. No obvious restrain effect on the mice bone marrow cells was observed. No obvious damage side effect on the mice bone marrow cell chromosome was observed by levocarnitine.

In human studies，levocarnitine is used to cure angina pectoris and heart failure. Few patients have some adverse reaction, such as nausea, uncomfortable stomach, dizziness. All these syndromes are slight, no need to stop taking medicine. They can continue to receive the therapy (refer to appendix D).

Some literatures said, l-carnitine is very well tolerated. 【19】 Few side effects have occurred during treatment with doses as high as 15g daily, other than infrequent diarrhea (Snyder et al. 1982; Waber et al. 1982) which is likely dose-related, or other gastrointestinal complaints. In an open study of 12 months duration conducted in more than 4000 patients with cardiac disease, gastralgia, nausea and diarrhea were the adverse effects reported most commonly, Attacking in 6%, 5%, 2%, respectively, of patients given oral l-carnitine 2g daily (Fernandes et al. 1985). Importantly, a myasthenia

gravis-like syndrome which has occurred in patients undergoing haemodialysis who were treated with dl-carnitine (Clair et al. 1984; De Grandis et al. 1980), has not been observed with l-carnitine therapy. Bazzato et al. (1981) postulated that the accumulation of the d-isomer of carnitine in uraemic patients may elicit a hemicholinium-like block.

The Expert Panel【appendix E】 convened by NEPG, independently and collectively, critically evaluated the data and information summarized above and concluded that the proposed use of Levocarnitine as an ingredient in foods and beverages, produced consistently with cGMP and meeting appropriate food grade specifications described herein, is safe.

They further concluded that the proposed use of Levocarnitine as a food ingredient is GRAS based on scientific procedures. It is also NEPG's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

Based on scientific procedures, Levocarnitine is GRAS under the intended conditions of use as a food ingredient, and therefore, Levocarnitine is exempt from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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[1]Institute for Eye Research, Sydney, New South Wales, Australia

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APPENDICES

Appendix A: Expert panel consensus statement concerning the generally recognized as safe status of levocarnitine as a food ingredient

Appendix B: Certificates of five consecutive representative batches of levocarnitine

Appendix C: Stability study data

Appendix D: Clinical study sponsored by NEPG

Appendix E: Experts' Curricula vitae

TABLETS

Table II.C.1-1 Specification of levocarnitine

Table II.C.3-1 Stress test

FIGURES

Figure II.B-1 Flow chart of manufacturing process

Figure IV.D.1-1 Biosynthesis of levocarnitine

Figure IV.D.1-2 Metabolism of levocarnitine

Appendix A Expert panel consensus statement concerning the generally recognized as safe status of levocarnitine as a food ingredient

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Levocarnitine as a food Ingredient

October 2010

As independent experts qualified by relevant experience and scientific training to evaluate the safety of food ingredients, we, the undersigned, Xue Hui (Health Supervision institute) Xie Tao (Center for disease control and prevention), Yu Hezhou, Huang Wenshu (Scientific & Technological Development Company of Northeast Pharmaceutical Group), were requested by Northeast Pharmaceutical Group Co., Ltd. (NEPG), as an Expert Panel (hereinafter referred to as the Panel) to evaluate the uses and use levels on the Generally Recognized as Safe (GRAS) status of Levocarnitine, under the conditions of intended use in conventional foods, beverage.

Previously, the safety and GRAS status of Levocarnitine for various intended food uses was critically evaluated by the Panel. The Panel concluded that the use of Levocarnitine at specified levels in the intended foods was safe and GRAS based on scientific procedures.

So far, levocarnitine, as the nutrient fortification substance, is listed in *National Standard of the People's Republic of China, Hygienic standard for the use of nutritional fortification substances in foods* (GB14880-1994). In which the use scope and dietary intake are determined. Levocarnitine is classified in the vitamin series, called vitamin BT.

INTENDED USES AND ESTIMATED EXPOSURE OF LEVOCARNITINE

The specified use scope and dietary intake in *National Standard of the People's Republic of China, Hygienic standard for the use of nutritional fortification substances*

in foods (GB14880-1994) are as below:

Chewing tablets, oral liquid, capsule: 250～600mg/tablet, piece, pill;

Milk powder: 300～400g/kg;

Fruit juice or fruit flavor beverage, milk beverage: 600～3000mg/kg;

Formula milk powder for children: 5~15 mg/100g;

Sports nutrition food: 1－4g/day

L-carnitine is synthesized endogenously from methylation of the amino acid L-lysine by S-adenosylmethionine (SAMe). Magnesium, vitamin C, iron, vitamins B3 and B6, and alpha-ketoglutarate – along with the cofactors re sponsible for creating SAMe (methionine, folic acid, vitamin B12, and betaine) – are all required for endogenous carnitine synthesis.

Levocarnitine also is present naturally in many foods, including fresh meats, and poultry, as well as milk and bread. Animal products like meat, fish, poultry, and milk are the best sources. Dairy products contain carnitine primarily in the whey fraction.
A well-balanced, non-vegetarian Western diet is estimated to provide 100-300 mg of L-Carnitine each day. The average non-vegetarian diet provides up to 100 mg L-carnitine daily, or up to 300 mg in high meat eaters. The richest sources are from meat, sheep muscle containing the most at around 207 mg/100 g, with milk, rice and bread being lesser sources. Carnitine homeostasis is maintained by absorption from dietary sources, a modest rate of biosynthesis and highly efficient reabsorption of carnitine in the kidney (Rebouche and Seim, 1998). Estimates of the amount of L-carnitine absorbed from the diet vary from about 30 - 40% (Harmeyer, 2000) to 54 - 87% (Rebouche and Seim, 1998).

DATA SUPPORTING THE SAFETY OF LEVOCARNITINE

The determination of the safety of Levocarnitine under the conditions of intended use is based on the results of published toxicological and human studies, the data obtained from the third party, Liaoning Province center for disease control and prevention, whom NEPG commissions to conduct the preclinical studies. We also reference the

information on the background dietary consumption of levocarnitine and its metabolic fate, as well as its presence endogenously in humans.

Data from preclinical study of levocarnitine (batch number DY017100142 produced by NEPG and tested by Center for disease control and prevention of Liaoning Province) on mice were reviewed for the purpose of assessing the safety and GRAS status of Levocarnitine, including an acute oral toxic test on mice, Micronucleus Test of Mice Bone Marrow Polychromatic Erythrocytes, and Salmonella typhimurium/mammal microsomal enzyme test (AMES test). These test results indicates that:

1. An acute oral toxicity test in mice: the female and male mice LD_{50}>15000mg/kg BW, which shows no toxicity of levocarnitine.
2. Micronucleus Test of Mice Bone Marrow Polychromatic Erythrocytes: the various dose groups are compared with control groups, there is no obvious difference on the micronucleus cell rate, and the test result is negative.
3. Salmonella typhimurium/mammal microsomal enzyme test (AMES test): the test result is negative.

The safety of levocarnitine also is supported by human studies that demonstrated its tolerability following oral administration. 【19】 Few side effects have occurred during treatment with doses as high as 15g daily, other than infrequent diarrhea (Snyder et al. 1982; Waber et al. 1982) which is likely dose-related, or other gastrointestinal complaints. In an open study of 12 months duration conducted in more than 4000 patients with cardiac disease, gastralgia, nausea and diarrhea were the adverse effects reported most commonly, Attacking in 6%, 5%, 2%, respectively, of patients given oral l-carnitine 2g daily (Fernandes et al. 1985). Levocarnitine produced by NEPG is used to cure angina pectoris and heart failure. Few patients have some adverse reaction, such as nausea, uncomfortable stomach, dizziness. All these symptoms are slight.

In addition, we also review certificates of analysis of five consecutive batches of levocarnitine according to USP 33 and FCC7 provided by the QC lab of NEPG. All the tested items meet the standard; the product has good quality and is safe.

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CONCLUSION

We, the Expert Panel, have independently critically evaluated the data and information summarized above and concludes that the proposed uses of Levocarnitine, meeting food-grade specifications and produced in accordance with current good manufacturing practices, are safe. We further conclude that Levocarnitine is Generally Recognized as Safe (GRAS) by scientific procedures for use in foods under the conditions of intended use described herein. It is our opinion that other qualified experts would concur with these conclusions.

Xue Hui (b) (6) Date 2010.10.22

Health Supervision institute of Liaoning Province, P.R.China

Xie Tao (b) (6) Date 2010.10.22

Center for disease control and prevention of Liaoning Province, P.R.China

Yu Hezhou, Huang Wenshu Date 2010.10.22

(b) (6)

Scientific & Technological Development Company of Northeast Pharmaceutical Group.

Appendix B Certificates of five consecutive representative batches of levocarnitine

东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

No. 37 Zhonggong Bei Street, Tiexi District, Shenyang, China. Post code: 110026 FAX: 024-22724536 TEL 024-22721818

CERTIFICATE OF ANALYSIS
LEVOCARNITINE (L-CARNITINE BASE) FCC

BATCH NUMBER	DY 017100191	**MANUFACTURE DATE**	Sep.8.2010
BATCH SIZE	1000 kg	**TEST DATE**	Sep.12.2010
QUANTITY	40 Drums	**RETEST DATE**	Sep.7.2012

	Analysis Items	Specifications	Analysis Results
1.	Characteristics	White crystal or white crystalline powder, hygroscopic	White crystalline powder
2.	Identification	Chemical Analysis: Positive IR Absorption: the spectrum obtained with the substance to be examined correspond with the spectrum obtained with the RS.	Positive Complied
3.	Assay(On Dry Basis)	97.0%~103.0%	98.4%
4.	Specific Rotation	-29.0°~-32.0°	-31.1°
5.	Acidity or Alkalinity (pH)	5.5~9.5	7.09
6.	Water Content	≤4.0%	1.17%
7.	Sodium	≤0.1%	0.0017%
8.	Chloride	≤0.4%	<0.4%
9.	Residue on Ignition	≤0.5%	0.06%
10.	Potassium	≤0.2%	0.0212%
11.	Lead	≤0.0001%	<0.0001%

We, **Northeast Pharmaceutical Group Co., Ltd.**, certify that this batch of **LEVOCARNITINE (L-CARNITINE BASE)** meets the requirements of **FCC7.**

Analysts (b) (6) (b) (6) **Checker** (b) (6) **Supervisor** (b) (6)

Final Batch Disposition

Approved **By:** (b) (6)

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东药集团
NEPG

东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

No. 37 Zhonggong Bei Street, Tiexi District, Shenyang, China. Post code: 110026 FAX: 024-22724536 TEL: 024-22721818

CERTIFICATE OF ANALYSIS
LEVOCARNITINE (L-CARNITINE BASE) FCC

BATCH NUMBER	DY 017100192	**MANUFACTURE DATE**	Sep.9.2010
BATCH SIZE	1000 kg	**TEST DATE**	Sep.13.2010
QUANTITY	40 Drums	**RETEST DATE**	Sep.8.2012

	Analysis Items	Specifications	Analysis Results
1.	Characteristics	White crystal or White crystalline powder, hygroscopic	White crystalline powder
2.	Identification	Chemical Analysis: Positive IR Absorption: the spectrum obtained with the substance to be examined correspond with the spectrum obtained with the RS.	Positive Complied
3.	Assay(On Dry Basis)	97.0%~103.0%	98.8%
4.	Specific Rotation	-29.0°~-32.0°	-31.2°
5.	Acidity or Alkalinity (pH)	5.5~9.5	7.17
6.	Water Content	≤4.0%	0.80%
7.	Sodium	≤0.1%	0.0011%
8.	Chloride	≤0.4%	<0.4%
9.	Residue on Ignition	≤0.5%	0.08%
10.	Potassium	≤0.2%	0.0230%
11.	Lead	≤0.0001%	<0.0001%

We, **Northeast Pharmaceutical Group Co., Ltd.**, certify that this batch of **LEVOCARNITINE (L-CARNITINE BASE)** meets the requirements of **FCC7.**

Analysts (b) (6) **Checker** (b) (6) **Supervisor** (b) (6)

Final Batch Disposition

Approved **By:** (b) (6)

000048

东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.
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CERTIFICATE OF ANALYSIS
LEVOCARNITINE (L-CARNITINE BASE) FCC

BATCH NUMBER	DY 017100193	**MANUFACTURE DATE**	Sep.12.2010
BATCH SIZE	1000 kg	**TEST DATE**	Sep.16.2010
QUANTITY	40 Drums	**RETEST DATE**	Sep.11.2012

	Analysis Items	Specifications	Analysis Results
1.	Characteristics	White crystal or White crystalline powder, hygroscopic	White crystalline powder
2.	Identification	Chemical Analysis: Positive IR Absorption: the spectrum obtained with the substance to be examined correspond with the spectrum obtained with the RS.	Positive Complied
3.	Assay(On Dry Basis)	97.0%~103.0%	98.8%
4.	Specific Rotation	-29.0°~-32.0°	-31.2°
5.	Acidity or Alkalinity (pH)	5.5~9.5	7.62
6.	Water Content	≤4.0%	0.48%
7.	Sodium	≤0.1%	0.0007%
8.	Chloride	≤0.4%	<0.4%
9.	Residue on Ignition	≤0.5%	0.05%
10.	Potassium	≤0.2%	0.0190%
11.	Lead	≤0.0001%	<0.0001%

We, **Northeast Pharmaceutical Group Co., Ltd.**, certify that this batch of **LEVOCARNITINE (L-CARNITINE BASE)** meets the requirements of **FCC7.**

Analysts (b) (6) (b) (6) **Checker** (b) (6) **Supervisor** (b) (6)

Final Batch Disposition

Approved 质检专用章 **By:** (b) (6)

000049

东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

No. 37 Zhonggong Bei Street, Tiexi District, Shenyang, China. Post code: 110026 FAX: 024-22724536 TEL: 024-22721818

CERTIFICATE OF ANALYSIS
LEVOCARNITINE (L-CARNITINE BASE) FCC

BATCH NUMBER	DY 017100194	**MANUFACTURE DATE**	Sep.13.2010
BATCH SIZE	1000 kg	**TEST DATE**	Sep.16.2010
QUANTITY	40 Drums	**RETEST DATE**	Sep.12.2012

	Analysis Items	Specifications	Analysis Results
1.	Characteristics	White crystal or white crystalline powder, hygroscopic	White crystalline powder
2.	Identification	Chemical Analysis: Positive IR Absorption: the spectrum obtained with the substance to be examined correspond with the spectrum obtained with the RS.	Positive Complied
3.	Assay(On Dry Basis)	97.0%~103.0%	98.6%
4.	Specific Rotation	-29.0°~-32.0°	-31.4°
5.	Acidity or Alkalinity (pH)	5.5~9.5	7.33
6.	Water Content	≤4.0%	0.49%
7.	Sodium	≤0.1%	0.0025%
8.	Chloride	≤0.4%	<0.4%
9.	Residue on Ignition	≤0.5%	0.05%
10.	Potassium	≤0.2%	0.0184%
11.	Lead	≤0.0001%	<0.0001%

We, **Northeast Pharmaceutical Group Co., Ltd.**, certify that this batch of **LEVOCARNITINE (L-CARNITINE BASE)** meets the requirements of **FCC7.**

Analysts (b) (6) **Checker** (b) (6) **Supervisor** (b) (6)

Final Batch Disposition

Approved **By:** (b) (6)

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东北制药集团股份有限公司
NORTHEAST PHARMACEUTICAL GROUP CO., LTD.

No. 37 Zhonggong Bei Street, Tiexi District, Shenyang, China. Post Code: 110026 FAX: 024-22724536 TEL: 024-22721818

CERTIFICATE OF ANALYSIS
LEVOCARNITINE (L-CARNITINE BASE) FCC

BATCH NUMBER	DY 017100195	**MANUFACTURE DATE**	Sep.14.2010
BATCH SIZE	1000 kg	**TEST DATE**	Sep.18.2010
QUANTITY	40 Drums	**RETEST DATE**	Sep.13.2012

	Analysis Items	Specifications	Analysis Results
1.	Characteristics	White crystal or white crystalline powder, hygroscopic	White crystalline powder
2.	Identification	Chemical Analysis: Positive IR Absorption: the spectrum obtained with the substance to be examined correspond with the spectrum obtained with the RS.	Positive Complied
3.	Assay(On Dry Basis)	97.0%~103.0%	98.7%
4.	Specific Rotation	-29.0°~-32.0°	-31.1°
5.	Acidity or Alkalinity (pH)	5.5~9.5	7.23
6.	Water Content	≤4.0%	0.53%
7.	Sodium	≤0.1%	0.0011%
8.	Chloride	≤0.4%	<0.4%
9.	Residue on Ignition	≤0.5%	0.05%
10.	Potassium	≤0.2%	0.0204%
11.	Lead	≤0.0001%	<0.0001%

We, **Northeast Pharmaceutical Group Co., Ltd.**, certify that this batch of **LEVOCARNITINE (L-CARNITINE BASE)** meets the requirements of **FCC7.**

Analysts (b) (6) **Checker** (b) (6) **Supervisor** (b) (6)

Final Batch Disposition

Approved **By:** (b) (6)

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Appendix C Stability study data

Data of long-term testing

Batch Number: DY080082; Temperature: 25±2°C; Humidity: 60±5% RH

Items	*Acceptance criteria*	*Test period*							
		0 month	*3 months*	*6 months*	*9 months*	*12 months*	*18 months*	*24 months*	*36 months*
		April 3 2008	July 3 2008	Oct. 5 2008	Jan. 7 2009	April 4 2009	Oct. 3 2009	April 8 2010	
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
Specific rotation	-29°~ -32°	-31.4°	-31.4°	-31.3°	-31.2°	-31.2°	-31.1°	-30.9°	
pH	5.5~9.5	7.61	7.60	7.58	7.56	7.58	7.56	7.50	
Water content	≤ 4.0%	0.44%	0.45%	0.38%	0.49%	0.52%	0.48%	0.49%	
Assay	97.0%~103.0%	98.6%	98.6%	98.7%	98.6%	98.5%	98.6%	98.6%	

000052

Batch Number: DY080083; Temperature: 25±2°C; Humidity: 60±5% RH

Items	*Acceptance criteria*	*Test period*							
		0 month	*3 months*	*6 months*	*9 months*	*12 months*	*18 months*	*24 months*	*36 months*
		April 3 2008	July 3 2008	Oct. 5 2008	Jan. 7 2009	April 4 2009	Oct. 3 2009	April 8 2010	
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
Specific rotation	-29°~ -32°	-31.3°	-31.3°	-31.3°	-31.3°	-31.4°	-31.0°	-30.8°	
pH	5.5~9.5	7.63	7.64	7.66	7.62	7.64	7.60	7.64	
Water content	≤ 4.0%	0.44%	0.45%	0.42%	0.46%	0.49%	0.48%	0.45%	
Assay	97.0%~103.0%	98.6%	98.4%	98.6%	98.6%	98.4%	98.7%	98.7%	

000053

Batch Number: DY080084; Temperature: 25±2°C; Humidity: 60±5% RH

Items	*Acceptance criteria*	*Test period*							
		0 month	*3 months*	*6 months*	*9 months*	*12 months*	*18 months*	*24 months*	*36 months*
		April 3 2008	July 3 2008	Oct. 5 2008	Jan. 7 2009	April 4 2009	Oct. 3 2009	April 8 2010	
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
Specific rotation	-29°~ -32°	-31.3°	-31.3°	-31.2°	-31.2°	-31.3°	-31.0°	-30.9°	
pH	5.5~9.5	7.59	7.58	7.56	7.54	7.57	7.55	7.50	
Water content	≤ 4.0%	0.42%	0.45%	0.45%	0.48%	0.51%	0.49%	0.49%	
Assay	97.0%~103.0%	98.4%	98.4%	98.5%	98.5%	98.3%	98.5%	98.8%	

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• **Data of accelerated testing**

Batch Number: DY080082; Temperature: 40±2°C; Humidity: 75±5% RH

Items	*Acceptance criteria*	*Test period*				
		0 month	*1 month*	*2 months*	*3 months*	*6 months*
		April 3 2008	May 3 2008	June 3 2008	July 3 2008	Oct. 5 2008
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive
Specific rotation	-29°~ -32°	-31.4°	-31.4°	-31.2°	-31.1°	-30.8°
pH	5.5~9.5	7.61	7.60	7.58	7.65	7.68
Water content	≤ 4.0%	0.44%	0.44%	0.55%	0.66%	0.70%
Assay	97.0%~103.0%	98.6%	98.4%	98.4%	98.5%	98.2%

Batch Number: DY080083; Temperature: 40±2°C; Humidity: 75±5% RH

Items	*Acceptance criteria*	*Test period*				
		0 month	*1 month*	*2 months*	*3 months*	*6 months*
		April 3 2008	May 3 2008	June 3 2008	July 3 2008	Oct. 5 2008
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive
Specific rotation	-29°~ -32°	-31.3°	-31.3°	-31.2°	-31.0°	-30.9°
pH	5.5~9.5	7.63	7.62	7.66	7.70	7.72
Water content	≤ 4.0%	0.44%	0.42%	0.56%	0.67%	0.69%
Assay	97.0%~103.0%	98.6%	98.4%	98.4%	98.3%	98.3%

000056

Batch Number: DY080084; Temperature: 40±2°C; Humidity: 75±5% RH

Items	*Acceptance criteria*	*Test period*				
		0 month	*1 month*	*2 months*	*3 months*	*6 months*
		April 3 2008	May 3 2008	June 3 2008	July 3 2008	Oct. 5 2008
Characters	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic	White crystalline powder, hygroscopic
Identifi-cation	Positive	Positive	Positive	Positive	Positive	Positive
Specific rotation	-29°~ -32°	-31.3°	-31.3°	-31.2°	-31.1°	-30.9°
pH	5.5~9.5	7.59	7.60	7.61	7.68	7.70
Water content	≤ 4.0%	0.42%	0.44%	0.55%	0.66%	0.69%
Assay	97.0%~103.0%	98.4%	98.4%	98.4%	98.3%	98.6%

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Appendix D Clinical study sponsored by NEPG

Materials and Methods

The levocarnitine is produced by Northeast Pharmaceutical Group Co., ltd.

We study the therapeutic effects of levocarnitine produced by NEPG on angina and heart failure in June 1994- April 1995.

Information source:

Unit	Angina		Heart failure		Total
	Placebo group	Levocarnitine group	Placebo group	Levocarnitine group	
The First Hospital of China Medical University	15	30	9	21	75
The Second Hospital of China Medical University	10	24	10	10	54
Beijing Friendship Hospital	10	13	8	9	40
Total	35	67	27	40	169

Method:

I. Observed objectives

I.A Angina pectoris

I.A.1 The patients have the typical angina pectoris symptoms, including fatigue, various types of unstable angina, excluding disease of aortic valve, cardiac muscle disease and other pains induced by other diseases.

I.A.2 Ischemic cardiogram change: at statistic status or angina attacks, the R wave of eletro-cardiogram is 0.08seconds after the J point, horizontal or down sloping ST segment depression ≥1mm, or ST segment elevation >1.0mm, or cardiogram exercise load test indicates positive, but it's necessary to exclude ST-T changer due to electrolyte turbulence or medicines factors.

I.A.3 Coronary arteriongraphy indicates that the blood vessel of patients have pathological narrow.: patients who possess standard 1 and own standard 2 or 3 at the same time, or when angina occurs, keep glonoin in the mouth, the symptom relieves within 3 min or have myocardial infarction disease history can be the candidates.

I.B Heart failure

I.B.1 Patients who have chronic cardiac insufficiency induced by various heart diseases (such as coronary heart disease, hypertension heart disease, Cardiac muscle pathological changes, rheumatic heart disease) or have class III or over class III cardiac functions.

I.B.2 Patients who have the following conditions will not be the candidates: acute cardiac failure, obvious insufficiency of hepatic and renal functions, hypotension, or shock, electrolyte turbulence, hypothyroid disease, anemia, pericardium disease, severe arrhythmia, lung block, pulmonary heart disease infection, infective endocarditis, or who are in the observation duration of another new medicine.

II. Requirements on observation

II. 1 During the process of taking levocarnitine, the former other medicines are still used, record the doses and usage of each medicines.

II. 2 Take levocarnitine based on the former therapeutic method, set up placebo-group whose patients should not be less than 1/3 of the total patients. The placebo should be same with levocarnitine in shape, usage.

II. 3 Dose and period of treatment: 10 ml/ampoule (containing 1g of levocarnitine), three times per day, 1g for each time; take orally, for one month. The usage and period of treatment of placebo is the same with that of levocarnitine.

III. Observed contents

III.A Angina pectoris

III.A .1 Change of symptom: Attacking times, extent, inducing fatigue intension of angina.

III.A .2 Daily consumption of glonoine before and after treatment.

III.A .3 Blood pressure, heart rate, rate-pressure-product, cardiac rhythm

III.A .4 Side effects

III.A.5 Test cardiogram when resting, angina attacks, movement electro-cardiogram

when necessary.

III.A.6 Routine blood test, blood platelet, routine urine test, liver function, kidney function, blood sugar, blood fat.

The above item 1-4 are recorded once a week before and after taking medicine; item 5-6 are recorded once before and after treatment.

III.B Heart failure

III.B.1 Clinical symptom and change on physical sign: dyspnea, cough, edema, heart rate, heart rhythm, blood pressure, body position, body weight, breath frequency, jugular vein, two lungs rale, liver size and heart function classification (based on NYHA standard).

III.B.2 Exercise capacity, exercise tolerance (6min walking distance)

III.B.3 Side effects of medicines

Record the above items once a week before and after taking medicine.

III.B.4 Chest PA, cardiogram, ultrasonic cardiogram when situation permitting.

III.B.5 Chemical test and biochemical items are same as III.A.6.

Record item 4-5 once before and after treatment.

IV Efficacy judgment criteria

IV.A Angina pectoris

Positive effect: Under the same labor intensity, no angina pectoris takes place or the Attacking times reduce over 80%, consuming of glonoine reduces over 80%.

Have effect: the Attacking times of angina pectoris and consuming amount of glonoine reduce 50-80%.

No effect: The attacking times of angina pectoris and consumption of glonoine reduce not more than 50%.

Aggravated: Attacking times of angina increases, aggravate the severity extent and duration time, as well as increase of consumption of angina.

IV.B Heart failure

Positive effect: Heart function improvement class II.

Have effect: Improve heart function class I

No effect: Not meeting with the above criteria.

V. Statistics: The data is compared with mean value ± standard deviation ($\bar{x}$±SD) by T test or chi-square test, $P<0.05$, there is obvious difference.

Results

I. Angina pectoris group

I.1 General Data, all the patients have coronary heart disease.

Table 1 General information of patients

Groups	Gender		Age	Type of angina			Combined other diseases		
	Male	female		unstable	fatigue	unclassified	Myocardial infarction	hypertension	diabetes
Placebo-group(36)	24	11	61±10	24	11		12	19	4
Levocarnitine group(67)	48	19	60.6±9.8	33	18	16	23	31	8

From the above information, we can see that there is no obvious difference, $P>0.1$.

I.2 Comparison of electro-cardiogram (ECG)

Table 2

Groups	Normal ECG	Ischemic ST-T change of ECG
Placebo-group(36)	5	30
Levocarnitine group(67)	9	58

There is no obvious difference between both groups, $P>0.1$.

I.3 Status when combining with other medicines

Both groups should be treated with anti-angina drugs, including nitrates, beta-blocker, calcium antagonists and other drugs. After taking levocarnitine for one month, reduce the dose of the anti-angina drugs on 5(14.3%) of 35 patients in placebo-group, 15 (22.4%) of 67 patients in levocarnitine group.

I. 4 Clinical effect: Evaluate the data based on the same criteria.

Table 3

	Positive effect	have effect	No effect	Total effective rate
Placebo-group(36)	3	19	13	62.86%
Levocarnitine group(67)	22	38	7	89.6%
P	P<0.01			

Besides the above evaluation of clinical effect, the Attacking times of angina pectoris in levocarnitine group are reduced more than that of placebo-group. See table 4.

Table 4

Attacking times of angina pectoris

	Attacking times		
	reduction	no change	increase
Placebo-group	23	10	2
Levocarnitine group	63	4	0
P	<0.01		

I. 5 Effect of ECG

The patients, whose former ECG are normal within both groups, the ECG still don't have change during the observation period. The patients, whose ECG have ischemic change, the ECG change after taking medicine. See table 5.

Table 5 Change of ECG

	Ischemic ST segment change			Ischemic T wave change		
	aggravation	no change	improvement	aggravation	no change	improvement
Placebo-group	1	9	6		15	8
Levocarnitine group		17	11		28	14

ST segment improvements in placebo-group and levocarnitine group are 37.5%, 39.3%, respectively. T-wave improvements in both groups are 34.6%, 33.4% respectively. There are not obvious differences between both groups.

I. 6 Comparison of heart rate and rate-pressure product (RPP) before and after taking medicines.

Table 6 Heart rate, RPP change

	Heart rate (times/min)		RPP	
	Before taking medicine	after taking medicine	Before taking medicine	after taking medicine
Placebo-group	79.3±12.2	72.1±9	10576.1±2458.8	9319.2±2079.3
Levocarnitine group	76.5±9.4	75.2±8.6	10103.4±2083.3	9229.9±1532.3
P	>0.5			

There is no obvious difference on heart rate, RPP before and after taking medicines within both groups. RPP reflects the oxygen consumption by cardiac muscle. The oxygen consumption is related to fatigue angina pectoris attacking. Due to this clinical study focus on unstable angina patients, therefore, RPP change is not obvious.

I. 7 Arrhythmia change

Comparison of the records of arrhythmia and 24hours dynamic ECG before and after treatment (excluding occasional premature beat).

Placebo-group: ventricular premature beat, 11 patients, after treatment, 4 of them are better; atrial premature beat, 7 patients, one of them are better; one patient with atrial tachycardia, is better after treatment; one patient of Atrial fibrillation, no change after treatment.

Levocarnitine group: 3 of 4 patients with ventricular premature beat are better; 4 of 7 patients with atrial premature beat are better; two atrial fibrillation patients, one doesn't change, the other paroxysmal one doesn't attack; one paroxysmal atrial tachycardia patient, paroxysmal atrial fibrillation attacks.

Due to few patients, it is difficult to determine the effect of levocarnitine on arrhythmia of angina pectoris patients.

I. 8 Adverse reaction

Placebo-group: one patient with dizziness.

Levocarnitine group: 2 patients with dizziness, 5 Gastrointestinal reaction patients

(nausea, uncomfortable stomach), in which one patient with diarrhea, two patients with hypodynamia.

After having the above reactions, one patient with gastrointestinal reaction has to stop treatment, the others don't need to stop taking medicine. The adverse reaction becomes better in the process of treatment.

I. 9 Chemical test

After both groups are treated, there is no abnormal situation on routine blood test, blood platelet, electrolyte, liver, kidney functions. In placebo-group, urine test of one patient is better; in levocarnitine group, urine test of 2 patients are better, the GPT decrease to normal level after taking levocarnitine, two patients; the BUN, Creatinine decrease to normal level after taking medicine, one patient.

Effect on blood fat: compare the effect of before and after taking treatment, there is no obvious change in placebo-group; 2 patients with Triglyceride decrease, one patient with total cholesterol decrease, but there are two and one patient increase in the above symptoms respectively. The left patients don't have the obvious change.

Effect on blood sugar: In placebo-group, two patients with blood sugar decrease, in levocarnitine group, two patients with blood sugar decrease, two patients increase slightly. The change on blood sugar and blood fat don't exclude the effect of dietary and other factor.

II. Heart failure group

II.1 Comparison of general information

Table 7 General information of patients

Groups	Gender Female male	Age	Classification of heart function II III IV	Disease cause Coronary heart disease	hypertension	rheumatic heart disease	cardiac muscle disease	pulmonary heart disease
Placebo-group(27)	17 10	61±14.3	2 14 11	14	4	3	6	
Levocarnitine group (40)	24 16	58.7±13.7	1 24 15	15	5	7	12	1

Before treatment, both groups have no obvious difference.

II.2 Combined medicines usage situation

Both groups take cardiotonic, diuresis, and vasodilator drugs. Comparison of before and after treatment, these drugs application don't change.

II.3 Curative effect

II.3.1 Change of heart function classification before and after treatment, see table 8.

Table 8

Groups		I	II	III	IV
Placebo-group(27)	Before treatment	3	2	14	11
	After treatment	3	10	9	8
Levocarnitine group (40)	Before treatment	0	1	24	15
	After treatment	2	26	9	3

Compare the results between groups, $P<0.05$. The heart function improvement in levocarnitine group is better than placebo-group.

II.3.2 Clinical curative effect according to heart function classification

Table 9 Clinical effect

Group	No effect	Have effect	Positive effect	Total effective rate
Placebo-group(27)	9	1 6	2	66.67%
Levocarnitine group (40)	4	31	5	90%

$P<0.02$

Both groups use cardiotonic, diuresis, and vasodilator drugs, but the levocarnitine group's clinical effect and improvement to heart function are better than the placebo-group.

II.3.3 Symptom and physical sign change

Table 10 Change on heart rate

	Before treatment	After treatment	P
Placebo-group(27)	91.9±21.7	80.6±12.5	
Levocarnitine group (40)	94.6±14.8	81.8±10.9	>0.05

Table 11 Symptom and physical sign change before and after treatment

		improvement	no change
dyspnea	Placebo-group	16	10
	Levocarnitine group	28	3
Lung rale	Placebo-group	9	13
	Levocarnitine group	18	9
edema	Placebo-group	9	8
	Levocarnitine group	18	4
hepatomegaly	Placebo-group	11	7
	Levocarnitine group	11	3

In placebo-group, 11 of 19 patients blood stasis is improved; in levocarnitine group, 9 of 11 patients blood stasis is improved.

II.3.4 Exercise capacity change: 6 min-walking distances is used to reflect Exercise capacity. Patients in bed or inconvenient movement are excluded this observation. In placebo-group, 13 of 16 patients exercise tolerance increase from 63.4±62.1meters to 175.3±150.2meters. In levocarnitine group, 9 of 14 patients exercise tolerance increase from 81.6±87.9meters to 215.6±183.8meters. There is no obvious difference ($P>0.05$) between both groups.

II.3.5 Effect on arrhythmia

In placebo-group, 2 patients with atrial premature beat, 5 patients with ventricular premature beat, 2 patients with paroxysmal atrial tachycardia, 5 patients with atrial fibrillation, after treatment, ventricular premature beat and paroxysmal atrial tachycardia decrease one patient respectively, one patient with atrial premature beat has paroxysmal atrial fibrillation, the other patients are not affected. In levocarnitine group, 3 patients with atrial premature beat, 4 patients with ventricular premature beat, 2 patients with paroxysmal atrial tachycardia, 16 patients with atrial fibrillation. After treatment, the patients with ventricular premature beat decrease 2 cases, the others are not affected. From the above information, it's difficult to determine the curative effect of levocarnitine on heart failure combined with arrhythmia.

II.4 Adverse drug reaction

In placebo-group, one patient has xerostomia, 3 patients have dizziness, one patient has

sleepiness, one patient has nausea and vomit.

In levocarnitine group, 7 patients have slight nausea or uncomfortable stomach (one case has vomit), one patient has diarrhea, 3 patients have xerostomia, 5 patients have dizziness.

Both groups patients don't need to stop using the placebo or curative drugs, some cases become better in the process of treatment. The patients with heart failure are easy to have gastrointestinal symptom in the process of treatment due to gastrointestinal blood stasis.

II.5 Chemical test

In placebo-group, one patient hemoleukocyte decreases from 7900 to 3800/mm3 after treatment. The others of both groups are not found with hemogram change (no change on erythrocyte, hemoleukocyte, blood platelet).

In placebo-group, three cases with positive urinary protein or suspectable positive are better after treatment; however, two cases become positive urinary protein. In levocarnitine group, urinary protein of 8 cases become better or disappear, no positive patients or symptom become heavier. But it should consider the relationship between heart failure with urinary protein and extent of heart failure becoming good or not.

In placebo-group, one case with BUN, Creatinine increase become normal level, one case with Creatinine increase slightly; GPT recovers to normal and increase slightly, one case respectively; in levocarnitine group, no abnormal change are observed on liver, kidney and dielectric.

Effect on blood fat: In placebo-group, comparison of before and after treatment, cases of with cholesterin, Triglyceride increasing and recovering to normal are 2 patients respectively. In levocarnitine group, no blood fat patients are increased. Neither group has abnormal change on blood sugar.

Discussion

Levocarnitine is one of components of animal foods, a natural substance which exists in meat or meat extract. It is related to the energy metabolism in animal body, plays an important role in fatty acid metabolism. It involves in cardiac muscle fat metabolism, protect ischemic cardiac muscle, improve energy metabolism of cardiac muscle to promote the cell of cardiac muscle to produce energy; increase cardiac contractility, decrease oxygen consumption of cardiac muscle.

1. This study shows that levocarnitine combined with other anti-angina pectoris drugs have the clinical effect on ischemic heart disease, angina pectoris, which is about 89.6%. they also have effect on unstable and fatigue angina pectoris, resulting in less attacks of angina pectoris.

2. In this clinical study, we supplement levocarnitine on 40 heart failure patients of different causes (39 patients with heart function over class III) besides cardiotonic, diuresis, and dilatation drugs. The total effective rate reaches to 90%, decrease heart rate, improve symptom and heart function, increase exercise capacity. So levocarnitine could be as routine adjuvant drugs applied in clinical study in treatment of heart failure. It indicates certain effect.

3. Few patients indicate gastrointestinal reaction after taking levocarnitine, main symptom as follows: nausea, uncomfortable stomach, dizziness. But all those symptoms are slight, these patients also can continue to be cured without stop taking levocarnitine. This drug has no harmful effect on Hemogram (blood routine examination, blood platelet), electrolyte, liver function. No obvious change on blood fat, blood sugar is observed due to only one month curative duration.

Appendix E Experts' curriculum vitae

1. Xie Tao: master degree in medicine of Peking Union Medical College, now he is employed in Center for Disease Control and Prevention. An assessor of Qualification Approval of national Laboratory, a member of Environmental Mutagen Society of China, a member of Chinese Nutrition Society.
He ever involved in the key subject of Liaoning Province *Toxicology And Function Study On North Cordyceps Sinensis*, the main thesis such as mutagenicity of hair restorer, the main testing items of SD rats-feeding for 30 days, study on seal oil capsule adjusting blood fat, etc.

2. Xue Hui: Chemistry bachelor degree of Bohai University. Now she is employed in Health Supervision institute of Liaoning Province. Since 1999, she is director of Council of Liaoning Province Nutrition Society; since 2005, she is director of Council of Liaoning Province Health laws Society.

3. Yu Hezhou: Master degree in medicinal chemistry of West China Pharmaceutical University. He is employed in Scientific & Technological Development Company of Northeast Pharmaceutical Group. He has been always engaged in study and development of new drug, clinical study and registration work since graduating from university. Beside new drug, he also is responsible for the study and registration work on food additives, such as levocarnitine, levocarnitine L-tartrate, magnesium ascorbyl phosphate and nutrition intensifying food.
The main thesis and study are as below:
Develop on new clinical use of carboprost suppository; levocarnitine; Study of toxic effects on kidney induced by anticancer agent of cisplatinum; primary discussion of the frequent problems occurred in clinical study of new drug; pharmacokinetics and bioavailability study on TRANILAST in human body, etc.

4. Huang Wenshu: Bachelor degree in Analytical chemistry, Fudan University. She is now employed in Scientific & Technological Development Company of Northeast

Pharmaceutical Group. She ever was trained on guidelines of new drug research technology, dissolution testing technology seminar, crystal pharmaceuticals study, drug impurities study, lab qualification approval during the year of 2005-2010. From year 2003 to 2010, she has the following invention patents: Manufacturing method of carboprost preparation absorbed via buccal mucosa; new manufacturing method for Magnesium ascorbyl phosphate; pilot trial technology system construction for new drug.

SUBMISSION END